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(54) Title: CONVERSION OF LAMININ 5 TO A MIGRATORY SUBSTRATE BY PROTEOLYSIS OF THE 'gamma'¿2 CHAIN THEREOF

(54) Titre: CONVERSION DE LAMININE 5 EN SUBSTRAT MIGRATOIRE PAR PROTEOLYSE DE LA CHAINE 'gamma'¿2 DE CELLE-CI

(57) Abstract

Conversion of laminin 5 to a migration-promoting molecule by treatment with a protease other than a matrix metalloprotease. The protease-treated laminin 5 is applied to a medical device to promote colonization by a desired cell type.

(57) Abrégé

La présente invention porte sur la conversion de laminine 5 en molécule stimulant la migration, par traitement faisant intervenir une protéase autre qu'une métalloprotéase matricielle. La laminine 5 traitée par protéase est appliquée sur un dispositif médical afin de stimuler la colonisation par un type de cellule désiré.

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(54) Title: CONVERSION OF LAMININ 5 TO A MIGRATORY SUBSTRATE BY PROTEOLYSIS OF THE γ_2 CHAIN THEREOF

(57) Abstract

Conversion of laminin 5 to a migration–promoting molecule by treatment with a protease other than a matrix metalloprotease. The protease-treated laminin 5 is applied to a medical device to promote colonization by a desired cell type.

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Description

CONVERSION OF LAMININ 5 TO A MIGRATORY SUBSTRATE BY PROTEOLYSIS OF THE γ_2 CHAIN THEREOF

Field of the Invention

The present invention relates to conversion of laminin 5 (LN5) to a cell migration-promoting substrate by proteases. More specifically, the invention relates to cleavage of the γ_2 chain of laminin 5.

Background of the Invention

Cell migration is a process which occurs during vital naturally occurring physiological processes such as wound healing, immune responses, bone repair, inflammation and the like. For example, stimulation of cell migration is desirable in promoting recruitment of epithelial cells to a wound site to facilitate healing of the wound, and in promoting migration of immune cells to sites of infection.

Laminins are heterotrimeric extracellular matrix proteins consisting of three subunits: α, β and γ. There are at least five known α subunits ($\alpha_1,\alpha_2,\alpha_3,\alpha_4,\alpha_5$), three known β subunits (β_1,β_2,β_3) and two known γ (γ_1,γ_2) subunits (Miner et al., J. Cell. Biol. 137:685-701, 1997). Laminin 5 (LN5) is an $\alpha_3\beta_3\gamma_2$ heterotrimer which is typically associated with epithelial cell adhesion and sometimes with hemidesmosome formation. The designation "laminin 5" was coined by Burgeson et al. (Matrix Biol. 14:209-211, 1994) to refer to a protein which is secreted into the culture medium by human keratinocytes and enhances keratinocyte attachment (Rousselle et al., J. Cell Biol. 114:567-576, 1991; International Publication Nos. WO92/17498 and WO94/05316). A similar protein was also identified by Carter et al. (Cell 65:599-619, 1991; International Publication No. WO95/06660) and called epiligrin. This protein is similar to the basement membrane glycoprotein recognized by the GB_3 antibody in human keratinocyte culture medium called nicein (Hsi et al., Placenta 8:209-217, 1987).

LN5 is also produced by 804G and NBT-II rat bladder carcinoma cells (U.S. Patent Nos. 5,541,106 and 5,422,264, hereby incorporated by reference). A human epithelial cell line, MCF-10A, produces a LN5 extracellular matrix which also induces hemidesmosome formation. This extracellular matrix protein is described in U.S. Patent No. 5,770,448, the entire contents of which are hereby incorporated by reference.

U.S. Patent Numbers 5,422,264 and 5,541,106 describe the isolation of rat LN5 and its ability to induce adhesion and hemidesmosome formation in epithelial cells. The purification of soluble LN5 is described in U.S. Patent No. 5,760,179, the entire contents of which are hereby incorporated by reference. U.S. Patent Nos. 5,510,263 and 5,681,587, the entire contents of which are hereby incorporated by reference, disclose the successful passaging of fetal and adult islet cells when plated on a rat LN5-coated substrate. U.S. Patent No. 5,672,361, the entire contents of which are hereby incorporated by reference, discloses the growth of pancreatic islet cells on human LN5-coated substrates. U.S. Patent No. 5,585,267, the entire contents of which are hereby incorporated by reference, discloses the growth of epithelial cells on trans-epithelial appliances coated with rat LN5. U.S. Patent Application Serial No. 09/145,387, the entire contents of which are hereby incorporated by reference, discloses the use of LN5 for treatment of bone defects.

LN5 is a matrix component of epithelial tissue basement membranes and plays an important role in the initiation and maintenance of epidermal cell anchorage to the underlying connective tissue. Cell interaction with elements of the extracellular matrix impacts their adherence, motility as well as protein and gene expression (Adams et al., Develop. 117:1183-1198, 1993). In intact, normal tissue, epithelial cells bind to extracellular matrix molecules which are organized into a complex multiprotein structure called the basement membrane. The major components of the basement membrane include type IV collagen, proteoglycans and laminins. LN5 plays an important role in establishing firm adherence of epithelial cells to the basement membrane since it is necessary for the assembly and maintenance of stable anchorage devices between epithelial cells and hemidesmosomes (Green et al., FASEB J. 10:871-880, 1996; Baker et al., J. Cell Sci. 109:2509-2520, 1996). LN5 is also expressed at the budding tips of invading tumor cell populations, i.e. at sites where cancer cells are undergoing cell division but where there are most likely no hemidesmosomes (Pyke et al., Am. J. Pathol. 145:782-791, 1994; Pyke et al., Cancer Res. 55:4132-4139, 1995).

Proteases play an important role in the remodeling of basement membranes during wound healing, metastasis and tumor invasion. These proteases may induce specific cleavage events that result in alteration of function. For example, specific cleavage of LN5 by matrix metalloprotease-2 (MMP2) was shown to induce migration of breast epithelial cells by cleaving the LN5 γ_2 subunit at residue 587, exposing a putative cryptic promigratory site on LN5 that triggers cell motility (Giannelli et al., Science 277:225-228, 1997). This cleavage converted LN5 to a molecule that mediates cell migration in addition to cell adhesion. However, the serine protease plasmin and another matrix metalloprotease, MMP9, were unable to cleave LN5 (Giannelli et al., supra.).

There is an ongoing need for promoting cell migration both in vitro and in vivo. The present invention addresses this need.

Summary of the Invention

One embodiment of the present invention is a method for converting laminin 5 to a migration-promoting substrate, **comprising the step of contacting said laminin 5 with a protease which releases all or part of domains III, IV and V of the γ_2 chain thereof**, with the proviso that said protease is not a matrix metalloprotease. Preferably, the protease is elastase or chymotrypsin. In one aspect of this preferred embodiment, the protease is a serine protease. Preferably, the serine protease is cathepsin G, coagulation factor Xa, cucumisin, endoproteinase Arg-C, endoproteinase Glu-C, kallikrein, subtilisin A, thrombocytin or trypsin. In another aspect of this preferred embodiment, the protease is a cysteine protease. Preferably, the cysteine protease is actinidin, calpain I, calpain II, cathepsin B or clostripain. In yet another aspect of this preferred embodiment, the protease is papain, bromelain, ficin, S. aureus V8 protease, cathepsin D or pepsin. Preferably, the laminin 5 is rat laminin 5. Alternatively, the laminin 5 is human laminin 5.

The present invention also provides **an isolated laminin 5 protein resulting from proteolysis of the $\gamma 2$ chain and release of all or part of domains III, IV and V therefrom**, with the proviso that the protease is not a matrix metalloprotease. Preferably, the protease is elastase or chymotrypsin. In one aspect of this preferred embodiment, the protease is a serine protease. Preferably, the serine protease is cathepsin G, coagulation factor Xa, cucumisin,

endoproteinase Arg-C, endoproteinase Glu-C, kallikrein, subtilisin A, thrombocytin or trypsin. In another aspect of this preferred embodiment, the protease is a cysteine protease. Preferably, the cysteine protease is actinidin, calpain I, calpain II, cathepsin B or clostripain. In yet another aspect of this preferred embodiment, the protease is papain, bromelain, ficin, S. aureus V8 protease, cathepsin D or pepsin. Preferably, the laminin 5 is rat laminin 5. Alternatively, the laminin 5 is human laminin 5.

Another embodiment of the present invention is a **method for promoting migration of cells onto a medical device**, comprising the steps of: contacting a medical device with an isolated laminin 5 protein resulting from proteolysis of the $\gamma 2$ chain and release of all or part of domains III, IV and V therefrom, with the proviso that said protease is not a matrix metalloprotease; and placing the medical device in the vicinity of the cells. Preferably, the medical device is contacted in vitro. Alternatively, the medical device is contacted in vivo. Advantageously, the cells are epithelial cells.

The present invention also provides a medical device or labware item to which an isolated laminin 5 large protein fragment resulting from proteolysis of laminin 5 in the hinge region of the $\gamma 2$ chain with a protease, with the proviso that the protease is not a matrix metalloprotease, has been applied.

Brief Description of the Figures

Figure 1 is a graph showing that cleavage of rat laminin 5 (rtLN5) with chymotrypsin or elastase results in conversion of reLN5 to a migratory substrate. Elastase or chymotrypsin-digested rtLN5 or intact control rtLN5 was coated onto pre-treated glass coverslips and used as a substrate for human gingival epithelial (GEH25) cells in a gold migration assay. GEH25 cells did not migrate on control intact rtLN5, but migrated on rtLN5 digested for 1, 2 or 3 hours with either chymotrypsin or elastase.

Figure 2 is a graph showing that chymotrypsin and elastase digested rat laminin 5 bind with identical coating efficiencies to glass coverslips compared to intact control rtLN5. The coating efficiency of digested or intact rtLN5 was determined by incubating coated and control glass coverslips with the LN5 α_3 chain-specific monoclonal antibody CM6. After incubation with a horseradish peroxidase (HRP)-coupled secondary antibody, the immune complex was developed with OPD substrate in citrate buffer and samples were read at 490 nm in a plate reader. There was no difference in coating efficiencies between protease-treated rtLN5 and control rtLN5.

Figure 3 is a graph showing the adhesion of human FGmet2 pancreatic carcinoma cells to tissue culture dishes coated with intact rtLN5, rtLN5 cleaved with chymotrypsin for 1 hour, or rtLN5 cleaved with elastase for 1 hour. Chymotrypsin or elastase-treated rtLN5 or intact rtLN5 were used to coat a 96-well plate in serial dilutions starting with 10 µg/ml. A suspension of FGmet2 cells (80,000/well) was incubated for 30 min at 37°C, washed, fixed, stained with crystal violet and solubilized in 1% SDS. Quantitation of attached cells was performed by reading the plates at 595 nm. The experiment was performed in triplicate. Cell adhesion was the same for all three substrates.

Detailed Description of the Preferred Embodiments

The present invention includes the observation that cleavage of the γ_2 chain of LN5 with elastase, chymotrypsin or endoproteinase Lys-c in the hinge region thereof, results in the release of all or part of domains III, IV and V and converts LN5 to a substrate that promotes cell migration as shown by migration of an epithelial cell line on immobilized elastase- or chymotrypsin-treated LN5 (Example 3, Fig. 1). The "hinge region" of the γ_2 chain of LN5 is the region between domains II and III thereof. The protease-cleaved LN5 also retains its adhesion-promoting properties (Fig. 3). Cleavage of rtLN5 or human LN5 (hLN5)with elastase or chymotrypsin results in release of a protein fragment having a molecular weight of about 70 kDa. The remainder of the LN5 molecule, having a molecular weight of about 380 kDa, promotes cell migration in addition to cell adhesion. Because MMP2 also cleaves the LN5 γ_2 chain in the hinge region, this indicates that this region is uniquely labile to proteolytic degradation. MMP2 and elastase have been implicated in the modification of basement membranes in processes of tissue invasion. Thus, the proteolytically sensitive area in the γ_2 chain may be important in modulating LN5 activity, and may be affected by different enzymes in distinct situations.

However, it has been determined that MMP2 only cleaves rtLN5, but not hLN5 (Example 5), indicating that another protease which does cleave LN5, such as elastase, is physiologically relevant in humans. Among the proteases examined, elastase may be the most physiologically relevant because it is present in wound fluid, breast cancer tumor extracts and in the metastatic human lung carcinoma cell line EBC-1.

Although the conversion of LN5 to a migration-promoting substrate by cleavage of LN5 with elastase, chymotrypsin and endoproteinase Lys-c is disclosed herein, the use of any non-matrix metalloprotease is also contemplated for this cleavage and conversion. Types of proteases contemplated for use in the present invention include serine proteases, cysteine proteases and other non-matrix metalloproteases. Examples of serine proteases include cathepsin G, coagulation factor Xa, cucumisin, endoproteinase Arg-C, endoproteinase Glu-C, kallikrein, subtilisin A, thrombocytin and trypsin. Examples of cysteine proteases include actinidin, calpain I, calpain II, cathepsin B and clostripain. Other proteases suitable for use in the present invention include papain, bromelain, ficin, S. aureus V8 protease, cathepsin D and pepsin. The peptides listed above are commercially available from many sources, for example Calbiochem, San Diego, CA and SIGMA, St. Louis, MO. The ability of any particular protease to cleave laminin 5, resulting in release of all or part of domains III, IV and V of the γ_2 chain thereof, and to convert LN5 from an adhesion-promoting to a migration-promoting substrate can be determined using the migration assay described in Example 3. The incubation conditions for any of these proteases are well known, and may be found, for example, in the manufacturer's instructions provided with the proteases.

The large fragment of LN5 resulting from protease cleavage of the γ_2 chain in the hinge region is used to coat the surface of a medical device to which migration and colonization of a particular cell type is desired. The device is then placed into contact with a patient, for example implanted into a patient, using conventional surgical techniques as described in Sabiston, D. C., Jr., M.D., Textbook of Surgery: the Biological Basis of Modern Surgical Practice, 15th ed., W.B. Saunders Co., Philadelphia, 1997). Such medical devices include, but are not limited to, surgical meshes,

catheters, metal rods, metal pins, artificial joints, dental implants, wires and pacemakers. For example, protease-digested LN5 is applied to a surgical mesh which is placed onto a burn site of a patient. The protease-digested LN5 stimulates migration of epithelial cells to the mesh which accelerates the healing process in the wounded area. Alternatively, protease-digested LN5 is applied to a surgical mesh and placed into a culture dish containing epithelial cells. The LN5 promotes migration of epithelial cells onto the mesh. The mesh is then placed onto the burn site. Thus, the cells can be contacted with the LN5-coated device either in vivo or in vitro. Protease-treated LN5 is purified using any conventional chromatographic method in which the small (cleaved) fragment of LN5 and the protease elute well separated from the large fragment (e.g., gel filtration chromatography). LN5 may also be purified as described in U.S. Patent No. 5,760,179.

For application to a medical device which is used in vivo, LN5 is is provided in a pharmaceutically acceptable excipient or diluent which are nontoxic to recipients at the dosages and concentrations employed. These pharmaceutical formulations can be prepared by conventional techniques, e.g. as described in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, 1985. Suitable carriers, excipients or diluents for the preparation of solutions and syrups include water, polyols, sucrose and glucose. These formulations can additionally contain preservatives, solubilizers, stabilizers, viscosity agents, wetting agents, emulsifiers, buffers, atioxidants and diluents.

Protease-digested LN5 is applied to medical device, including transepithelial appliances and other shaped articles as disclosed in U.S. Patent No. 5,585,267, by either passive absorption or covalent linkage. Passive adsorption can be accomplished in a variety of ways, e.g. by immersion of an article in a LN5-containing solution, spraying the article with the solution, and the like. Alternatively, LN5 may be covalently linked to the medical device using any of a variety of well known cross-linking agents which target different chemical groups on proteins, including amino, carboxyl, sulfhydryl, aryl, hydroxyl and carbohydrate groups. All of the cross-linking reagents described below are available from Pierce Chemical Co. (Rockford, IL) and described in the Pierce catalog. Other cross-linking reagents are well known in the art, and may be available from other suppliers. There are two main types of cross-linking agents: homobifunctional and heterobifunctional. Homobifunctional cross-linkers have at least two identical reactive groups and can target primary amine or sulfhydryl groups. Examples of homobifunctional cross-linking reagents which target amine groups include dimethyl 3,3,-dithiobispropionimidate 2HCl (DTBP) and disuccimidyl suberate (DSS). Sulfhydryl-specific reagents include bismaleimidohexane (BMH) and 1,4-di-[3'-2'-pyridyldithio(propinamido)butane] (DPDPB). The main disadvantages of such homobifunctional cross-linkers are self-conjugation, intramolecular cross-linking and/or polymerization.

Heterodifunctional cross-linkers contain two or more different reactive groups that allow for sequential conjugations with specific groups of proteins, minimizing undesirable polymerization or self-conjugation. Heterodifunctional cross-linkers which react with primary or secondary amines include imidoesters and N-hydroxysuccinimide (NHS)-esters such as siccimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and succimidyl-4-(p-maleimidophenyl)-butyrate (SMPB). Cross-linkers which react with sulfhydryl groups include

maleimides, haloacetyls and pyridyl disulfides. Carbodiimide cross-linkers couple carboxyls to primary amines or hydrazides, resulting in formation of amide or hydrazone bonds. One widely used carbodiimide cross-linker is 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride).

The choice of cross-linking agent is dependent upon the surface chemistry of the article to which the LN5 is to be attached. A surface can be chemically modified or manufactured to contain particular reactive groups using methods well known in the art. For example, substrates can be manufactured which contain epoxide groups using methods well known in the art. In some instances, surface activation or modification (e.g., by plasma discharge, corona discharge, electron beam, RF energy, laser or strong acid or base treatment) may facilitate attachment of cross-linking agent.

In a preferred embodiment, the amount of LN5 applied to a medical device is between about 0.01 $\mu g/cm^2$ and 100 $\mu g/cm^2$. In a more preferred embodiment, the amount of LN5 to the medical device is between about 0.1 $\mu g/cm^2$ and 10 $\mu g/cm^2$. To attain these amounts, the device is immersed in or sprayed with LN5 solutions having concentrations of between about 0.001 mg/ml and 100 mg/ml, more preferably between about 1 mg/ml and about 10 mg/ml.

Another embodiment of the present invention is a medical device, including transepithelial appliances and other shaped articles as disclosed in U.S. Patent No. 5,585,267, the entire contents of which are hereby incorporated by reference, to which the protease-cleaved LN5 of the invention has been applied.

Protease-cleaved LN5 may also be used in research and diagnostics. For example, the protease-cleaved LN5 of the invention may be applied to any conventional labware item such as a tissue culture dish, microtiter plate, flask, bioreactor, slide, and the like, to which it induces migration of cells applied to or contained within the labware item. Another embodiment of the invention is a labware item to which protease-cleaved LN5 has been applied.

Another embodiment of the present invention is non-metalloprotease-digested LN5 which is cleaved in the hinge region of the γ_2 chain. Cleavage of LN5 results in release of a small protein fragment containing all or part of domains III, IV and V of the γ_2 chain of the LN5 heterotrimer. The resulting migration-inducing large fragment of LN5 is itself a novel molecule because the cleavage which occurs by using a non-matrix metalloprotease is not at the same site as that induced by the MMP2 of Giannelli et al., supra.

Example 1

MMP2 cleaves rtLN5

rtLN5 was purified from 804G rat bladder carcinoma cell conditioned medium as described in U.S. Patent No. 5,760,179. Purified rtLN5 was incubated with 2 units or 0.2 units MMP2 (gelatinase 72 kD; Boehringer Mannheim, Indianapolis, IN) for 18 hours at 37°C. The digested samples were analyzed by 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under both non-reducing and reducing conditions, and stained with Coomassie Brilliant Blue. Under non-reducing conditions, association of the three rtLN5 subunits is preserved and the LN5 heterotrimers resolve as a doublet. Under these conditions, exposure of rtLN5 to MMP2 results in release of an

approximately 65 kD fragment. Under reducing conditions, the γ_2 150 kD and γ100 kD polypeptide chains are converted into an 80 kDa fragment. The α_3 and β_3 polypeptides are not affected by MMP2 treatment.

Example 2

Cleavage of rtLN5 with chymotrypsin and elastase

rtLN5 was incubated with elastase (1:10 w/w) or chymotrypsin (1:100 w/w) for 1, 2 or 3 hours at 37°C and analyzed by 6% SDS-PAGE. The gel was transferred to nitrocellulose and analyzed by Western blotting using a rtLN5 polyclonal antiserum. The results showed the disappearance of the γ_2 150 kD and γ_2 100 kD polypeptides, and appearance of a polypeptide of approximately 80 kD. Thus, cleavage of rtLN5 occurs in the same region using elastase and chymotrypsin as with MMP2. Western blot analysis using the γ_2 chain-specific antiserum J20 showed that the 80kD polypeptide was a γ_2 chain digestion product.

Example 3

Conversion of rtLN5 to a migratory substrate

Elastase- and chymotrypsin-digested rtLN5 or intact control rtLN5 was coated onto pre-treated glass coverslips and used as a substrate for primary human gingival epithelial (GEH25) cells in a gold migration assay. Briefly, glass coverslips were washed with 100% ethanol, air dried and briefly dipped into 1% bovine serum albumin (BSA). After drying, coverslips were transferred into a 24-well plate and coated with intact, proteolytically processed LN5 or 3% BSA at 4°C overnight. A 1.4 mM colloidal gold/12 mM sodium carbonate solution was prepared by boiling, followed by addition of formaldehyde to a final concentration of 0.01%. Each coverslip was incubated with 500 µl colloidal gold solution for several hours and subsequently transferred to a new plate. GEH25 cells were seeded into each well (3,000/well) and allowed to migrate for 18 hours at 37°C in 5% CO_2. The cells were fixed in 3% paraformaldehyde in phosphate buffered saline (PBS) and pictures were taken. Photographs were scanned and a pixel analysis was done evaluating cleared areas generated by cell migration tracks versus the total area. Migration was evaluated by pixel analysis expressed as percentage of cleared areas generated by cell migration tracks versus the total area.

GEH25 cells did not migrate on control rtLN5, but migrated on rtLN5 digested for 1, 2 or 3 hours with either chymotrypsin or elastase (Figure 1).

Example 4

Binding efficiencies of chymotrypsin- and elastase-digested rtLN5

The coating efficiency of chymotrypsin- or elastase-digested or intact rtLN5 was determined by incubating coated and control glass coverslips with the LN5 α_3-specific monoclonal antibody CM6. After incubation with a horseradish peroxidase-coupled secondary antibody, the immune complex was developed with OPD substrate in citrate buffer. The results show that chymotrypsin- and elastase-digested rtLN5 bind with identical coating efficiencies to the glass coverslips compared to control rtLN5.

Example 5

MMP2 cleaves rtLN5, but not human LN5

rtLN5 and hLN5, purified from the human epithelial cell line MCF 10A using the method described in U.S. Patent No. 5,760,179, were digested under identical conditions using either 2 units or 0.2 units of MMP2 (gelatinase 72 kD, Boehringer Mannheim) for 18 hours at 37°C. The digested samples were analyzed by 6% SDS-PAGE and stained with Coomassie Brilliant Blue. While the rtLN5 was cleaved as described in Example 1, cleavage of hLN5 did not occur.

Example 6

Cleavage of hLN5 with chymotrypsin and elastase

LN5 was purified from MCF 10A cells and exposed to various concentrations of elastase or chymotrypsin, incubated for 1 hour at 37°C, analyzed by SDS-PAGE under reducing and non-reducing conditions and stained with Coomassie Brilliant Blue. Elastase and chymotrypsin induce cleavage of the γ_2 100 kD polypeptide chain and generate fragments of approximately 75 kD and 65 kD, respectively.

Example 7

Protease-cleaved LN5 retains adhesion-promoting activity

Chymotrypsin- or elastase-treated rtLN5 or intact LN5 were used to coat a 96-well plate in serial dilutions starting with 10 µg/ml. for attachment, a suspension of FGmet 2 cells (80,000/well) was incubated for 30 min at 37°C, washed, fixed, stained with crystal violet and solubilized in 1% SDS. Quantitation of attached cells was performed by reading the plates at 595 nm. All experiments were performed in triplicate. The results show that all three substrates promoted promoted the same relative amount of cell attachment (Fig. 3)

Example 8

Promotion of epithelial cell migration in vivo

A standard surgical mesh obtained from, for example, Johnson & Johnson Medical, Inc., is immersed in a pharmaceutically acceptable solution of hLN5 (5 mg/ml) purified from human MCF-10A epithelial cells as described in U.S. Patent No. 5,760,179, which has been cleaved with neutrophil or pancreatic elastase. The mesh is allowed to dry, and placed onto a laceration of a patient under sterile conditions. The elastase-cleaved hLN5 facilitates healing of the laceration by promoting migration and adhesion of epithelial cells at the wound site.

It should be noted that the present invention is not limited to only those embodiments described in the Detailed Description. Any embodiment which retains the spirit of the present invention should be considered to be within its scope. However, the invention is only limited by the scope of the following claims.

Claims

WHAT IS CLAIMED IS:

1. A method for converting laminin 5 to a migration-promoting substrate, comprising the step of contacting said laminin 5 with a protease which cleaves said laminin 5 in the hinge region of the $\gamma 2$ chain thereof, resulting in release of all or part of domains III, IV and V, with the proviso that said protease is not a matrix metalloprotease.

2. The method of Claim 1, wherein said protease is elastase or chymotrypsin.

3. The method of Claim 1, wherein said protease is a serine protease.

4. The method of Claim 3, wherein said serine protease is selected from the group consisting of cathepsin G, coagulation factor Xa, cucumisin, endoproteinase Arg-c, endoproteinase Glu-c, kallikrein, subtilisin A, thrombocytin, trypsin.

5. The method of Claim 1, wherein said protease is a cysteine protease.

6. The method of Claim 5, wherein said cysteine protease is selected from the group consisting of actinidin, calpain I, calpain II, cathepsin B, clostripain.

7. The method of Claim 1, wherein said protease is selected from the group consisting of papain, bromelain, ficin, S. aureus V8 protease, cathepsin D andpepsin.

8. The method of Claim 1, wherein said laminin 5 is rat laminin 5.

9. The method of Claim 1, wherein said laminin 5 is human laminin 5.

10. An isolated laminin 5 large protein fragment resulting from proteolysis of laminin 5 in the hinge region of the $\gamma 2$ chain with a protease, with the proviso that said protease is not a matrix metalloprotease.

11. The method of Claim 10, wherein said protease is chymotrypsin or elastase.

12. The method of Claim 10, wherein said protease is a serine protease.

13. The method of Claim 12, wherein said serine protease is selected from the group consisting of cathepsin G, coagulation factor Xa, cucumisin, endoproteinase Arg-c, endoproteinase Glu-c, kallikrein, subtilisin A, thrombocytin and trypsin.

14. The method of Claim 10, wherein said protease is a cysteine protease.

15. The method of Claim 14, wherein said cysteine protease is selected from the group consisting of actinidin, calpain I, calpain II, cathepsin B andclostripain.

16. The method of Claim 10, wherein said protease is selected from the group consisting of papain, bromelain, ficin, S. aureus V8 protease, cathepsin D and pepsin.

17. The method of Claim 10 wherein said laminin 5 is rat laminin 5.

18. The method of Claim 10, wherein said laminin 5 is human laminin 5.

19. A method for promoting migration of cells onto a medical device, comprising the steps of:

contacting a medical device with an isolated laminin 5 protein resulting from proteolysis of laminin 5 in the hinge region of the $\gamma 2$ chain thereof to release all or part of domains III, IV and V therefrom, with the proviso that said protease is not a matrix metalloprotease; and

placing said medical device in the vicinity of said cells.

20. The method of Claim 19, wherein said medical device is contacted in vitro.

21. The method of Claim 19, wherein said medical device is contacted in vivo.

22. The method of Claim 19, wherein said cells are epithelial cells.

23. A medical device or labware item to which an isolated laminin 5 large protein fragment resulting from proteolysis of laminin 5 in the hinge region of the $\gamma 2$ chain with a protease, with the proviso that said protease is not a matrix metalloprotease, has been applied.

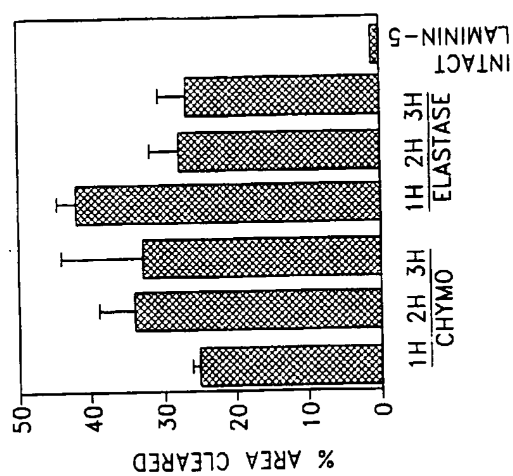
% AREA CLEARED
0
10
20
30
40
50
1H 2H 3H
CHYMO
1H 2H 3H
ELASTASE
INTACT
LAMININ-5

FIG.1

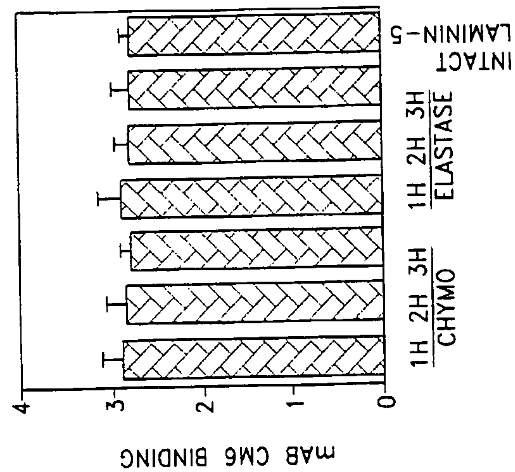
mAB CM6 BINDING
0
1
2
3
4
1H 2H 3H
CHYMO
1H 2H 3H
ELASTASE
INTACT
LAMININ-5

FIG.2

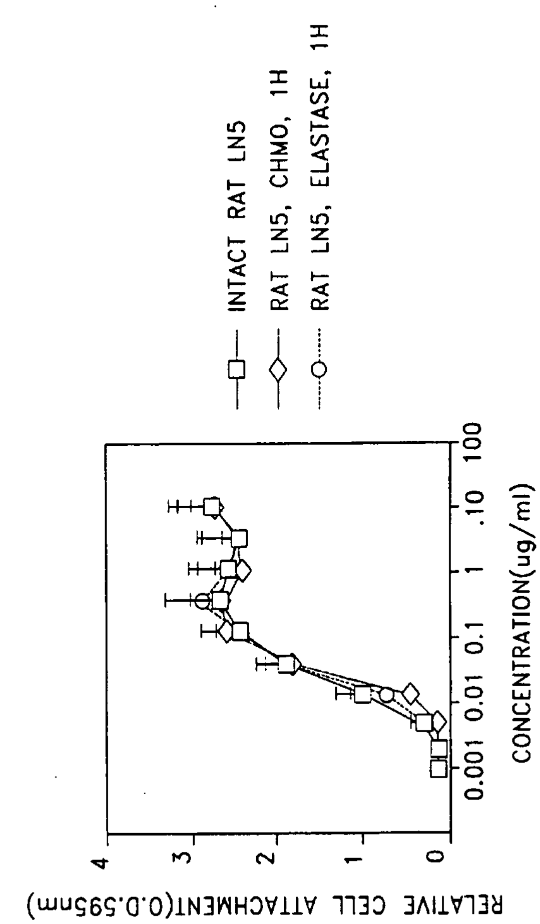
INTACT RAT LN5
RAT LN5, CHMO, 1H
RAT LN5, ELASTASE, 1H
RELATIVE CELL ATTACHMENT(O.D.595nm)
0
1
2
3
4
CONCENTRATION(ug/ml)
0.001
0.01
0.1
1
.10
100

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29433

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 5/00; A61B 17/00;A61B 17/04
US CL : 435/240; 535/240; 435/325; 606/151;128/334
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240; 535/240; 435/325; 606/151;128/334

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, STN

laminin 5, migration, cell matrix, protease, surgical mesh

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	ONO, Y. et al. Clinicopathologic Significance of Laminin-5 Gamma 2 Chain Expression in Squamous Cell Carcinoma of the Tongue. Cancer. 01 June 1999, Vol. 85, No. 11, pages 2315-2321, see entire document.	1-9
X	GIANNELLI, G. et al. Induction of Cell Migration by Matrix Metalloprotease-2 Cleavage of Laminin-5. Science. 11 July 1997, Vol. 227, No. 5323, pages 225-228, see entire document.	1-9 and 19-22
X	US 5,541,106 A (JONES) 30 July 1996 (30-07-96), see entire document.	1-9
X - Y	US 5,422,264 A (QUARANTA et al.) 06 June 1995 (06-06-95), see entire document.	1-9 ------ 19-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
22 MARCH 2000

Date of mailing of the international search report
25 APR 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29433

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,770,448 A (JONES et al.) 23 June 1998 (23-06-98), see entire document.	1-9 and 19-22
X	US 5,585,267 A (JONES et al.) 17 December 1996 (17-12-96), see entire document	19-22
X	US 5,292,328 A (HAIN et al.) 08 March 1994 (4-3-94), see entire document.	23
X	US 4,452,245 A (USHER) 05 June 1984 (05-06-84), see entire document	23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29433

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-9, drawn to Methods of converting laminin 5 to a migration-promoting substrate.
Group II, claim(s) 19-22, drawn to methods for promoting cell migration of cells onto a medical device.
Group III, claim(s) 23, drawn to a medical device or labware.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the categories related thereto. Accordingly, the main invention (Group I) comprises the first recited process, method for converting laminin 5 to a migration-promoting substrate. Further pursuant to 37 C.F.R. 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of the products and methods accordingly defines a separate invention.